U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Ocean Service
Office of Response and Restoration
Coastal Protection and Restoration Division
c/o EPA Region X (ECL-117)
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Dear Chip and Eric:

This letter provides **NOAA's comments, submitted in DRAFT, on the Round 3 Lamprey Ammocoete Toxicity Testing Field Sampling Plan Draft**. We appreciate LWG's efforts to produce document in a time-sensitive manner. The document, prepared by Windward Environmental LLC for the Lower Willamette Group, is dated September 29, 2006. NOAA recognizes that some of our comments, as herein submitted, may be more relevant to the QAPP. However, because the QAPP is not yet published, we are forwarding all of our comments on this effort at the present time in draft form. Please let us know if you need clarification or if you have any questions or input regarding which of these comments may be more appropriately deferred to the QAPP. We look forward to discussing this with you further in the very near future.

General Comments

NOAA notes that the FSP objectives, as currently described, specify establishing proper methods for collection, holding, and testing lamprey ammocoetes, but the text does not necessarily include detailed plans for such protocol development. We assume these details are forthcoming. In any case, the final version of the FSP should either include the detailed plans or specify the relevant document(s) where these plans are or will be described. If our assumption is incorrect, the objectives probably need to be redefined (e.g., test one protocol method, which may or may not be successful) or the procedures need to be expanded upon to allow for protocol development. Finally, the protocols should still provide some basic metrics used to define both the health of the organism.

Specific Comments

1. Page 1, Section 1.0: There is no introductory text regarding lamprey characteristics. Even if this FSP is not intended to be a stand-alone document, it would be worth including a few sentences about lamprey that influence the study objectives and/or methodology; e.g., anadromous life cycle, preferred substrate, burrowing and emergence time frames, filter feeding activity, temperature requirements, importance to tribal subsistence, population decline, etc.

2. Page 1, Section 1.1.1, bullets: The first three bullets of the stated objectives involve establishing proper methods for lamprey collection, holding, and exposure systems. These items involve protocol development, which (since established protocols are lacking) should be the main focus of Phase I. However, such development (e.g., testing with varying parameters) is not addressed in the field sampling procedures (see general comments above).

3. Page 4, Section 2.2: What degree of accuracy and precision will the GPS unit obtain and how will they be determined? (This may also be addressed in the QAPP). Also, last sentence is missing a period.

4. Page 5, Section 2.31, second bullet set: Site conditions should be noted in the field logbook, including habitat, substrate type, water quality, etc., and if possible, density of ammocoetes within a specified collection area.

5. Page 10, Section 3.1:

    a. First paragraph: Why the Siletz River? Indicate if the goal is to obtain specimens from a "clean" river, in the same/nearby drainage system, etc. This may also be an opportunity to describe the water temperature and other physical habitat characteristics that influence ammocoete abundance and, thus, sampling locations.
    b. First paragraph: Why "up to 800 ammocoetes"? The minimum number of organisms to collect needs to be stated, e.g., how many ammocoetes are deemed reasonable to test holding and acclimation procedures; similarly, a full test series might include 5 concentrations plus 1 control x 2 repetitions x 5 organisms per test x 6 chemicals = a total of 360 organisms. The number of organisms may also depend on the range of individual sizes collected.
    c. Second paragraph: Although there is evidence of readily collecting ammocoetes by electrofishing (Bayer et al., 2001), please explain the technique in more detail (e.g., the double-electroshock method seems overly stressful to the ammocoetes as well as potentially difficult). How will the sampler know when the ammocoetes have emerged from the sediment to apply the second shock (if turbid), and then how will those stunned specimens be collected from the water (very fine mesh net)? Since Phase I involves development of collection methods (see general comments above), other

options should be presented (e.g., grab sampling that collects and transports the ammocoetes within their native substrate, etc.).

d. Third paragraph: What are "representative individuals" and how are they selected? If the organisms are not measured, how will the test samples maintain and demonstrate comparable size and uniformity? Is a surplus of collected ammocoetes really expected to allow size selection? Some size-range limits should be set, however, to ensure that only a reasonable range is selected for testing.
e. Fourth paragraph: The last sentence states that "Ammocoetes will not be identified to the species level but left at genus level (*Lampetra* spp.)." More information should be included to support and clarify this rationale. We recommend including language along the following lines: "It is possible that up to four species may be present and, therefore, may be collected in the sampling area(s). Apparently, genetic analysis is the only known method for consistently and accurately identifying species among ammocoetes, though this is not deemed to be a practical method in the context of this effort. Furthermore, it is likely that because each of these four species are members of the same genus, the modes of action of various contaminants will be similar across species, as will species sensitivity to various contaminant concentrations. Finally, there are unlikely to be other life forms that might be present and that could subsequently be confused with the *Lampetra spp.*
f. Fifth paragraph: Since future sampling may occur in several watersheds, identify the criteria for selection of such watersheds / sampling areas.

6. Page 11, Section 3.2: Since this study should involve development of methodology, how will an appropriate substrate in which the ammocoetes are transported be selected? Do we know how they will react, for example, to being introduced to new sediment (sterile sand)? Maintaining the natural conditions may be preferred during transport (assuming no unknown contamination in the source material).

7. Page 12, Section 4.1:

   a. First paragraph: The introduction (Section 1.1.1) lists specific goals for the holding phase, but the discussion in Section 4.1 does not appear to provide any specific approach toward meeting those goals, e.g., varying temperature or feeding in a consistent manner among different holding chambers, and varying the size of the holding chambers/density of fish. Only one approach is specified, which appears to be obtained from standard protocols for toxicity testing of fathead minnows and/or rainbow trout (USEPA, 2002).
   b. First paragraph: How was the water hardness selected? Will the water body in which the organisms are collected be measured so that conditions can be replicated in the laboratory? Although other parameters not listed may be detailed in the QAPP (e.g., DO), they should be recognized and mentioned. Also, the type and source of water used in the study should be described.

c. First paragraph: Can't the feeding rate be at least estimated based on the size range of the ammocoetes?
d. Second paragraph: How will the health of the ammocoetes be determined during holding, e.g., regular weighing; swimming, burrowing, avoidance, or other responses; respiration rate; other sub-lethal effects; or simply by looking for dead fish?

8. Page 12, Section 4.2: According to Section 1.1.1, one goal of Phase 1 is to determine an appropriate test protocol. It is not clear in this section whether such protocol development is planned. Please describe in more detail how the Phase 1 water toxicity data will be used. In addition, there should be a size range identified for the chambers, presumably based upon the range of organism size, and this should be identified in Section 3. Randomization should also be applied when dividing the organisms between chambers to avoid size bias (e.g., so that not all of the smallest specimens are in one chamber). Please include language to this effect.

9. Page 13, first paragraph: The number of chemical concentrations to be tested should be determined and identified in this document (e.g., 3, 4, or 5?) – this will help in planning the sample collection – as well as the sample volume required to test each of the concentrations, e.g., why a minimum of 5 individuals per sample? Again, identify what constitutes "healthy ammocoetes." Same comments on husbandry as Section 4.1 above (e.g., describe source water).

10. Page 13, second paragraph: Although the chemicals to be tested do represent various modes of action, as suggested by the USEPA, it may be necessary to focus on just one, depending on the number of organisms used and the number of variations on the holding and testing protocols conducted during method development, followed by additional range-finding with the other compounds during the preliminary stages of Phase 2. If, for example, ammocoetes are found to be extremely sensitive to copper (a well-tested substance, easy to introduce into a laboratory system), the same relative sensitivity may be assumed as a starting point (relative to existing LC50 data) for the other compounds.

11. Page 13, Section 4.2, second paragraph: The document states "The concentrations of the six chemicals (i.e., copper, naphthalene, pentachlorophenol, lindane, diazinon, and aniline) used in the range-finding tests will be selected by the laboratory based on best professional judgement and LC50 values for other fish presented in the literature." It is important for the success of this study that appropriate concentration ranges be identified efficiently and expeditiously. NOAA suggests, therefore, that EPA and/or its partners should have the opportunity to provide input on the initial target concentration ranges when and as appropriate, rather than placing the burden for this determination exclusively on the shoulders of the selected labratory. (NOAA is not prepared to provide such input at the present time.)

12. Page 14, first paragraph: Similar to Phase 1, will there be any tests/observations of stress beyond death, e.g., poor swimming, lack of avoidance response, etc.? What will be the metrics for health at the start of the test? If there is a substantial range in sizes of the available fish, will there be a randomization procedure to ensure against size bias in the test chambers?

13. Page 14, first paragraph: How will the decision be made to do the side-by-side tests with ammocoetes and rainbow trout instead of adjusting the exposure concentration ranges? In addition, how would that decision be made by spring of 2007 (as required to provide the protocol to USEPA at that time)?

14. Page 14, second paragraph: It would be helpful if the authors would explain why the range-finding tests would be repeated as an option. Are they important or not?

15. Page 14, Section 4.5: The text should state specifically what water samples would be collected and submitted for laboratory confirmation testing, e.g., before and after exposure, from every test chamber, etc. The volumes of water required for the analyses is quite large (page 17, Table 5.1) and individual tests may not provide that much water.

16. Page 18, Section 5.2: It would be helpful if the text clarified how the concentration goals and reporting and detection limits compare to the possible exposure ranges in the testing.

17. Page 18, Section 5.3: Same comment as Section 5.2 regarding detectable concentrations of the analytes – e.g., if a compound is detected only at the ACG, some of the lowest LC50 values (such as those for lindane and pentachlorophenol) that are being tested may not be detected.

18. Page 18-19, Section 5.3: If the FSP retains the testing approach as specified, Sections 4.5 and 5.3 should be made specific in terms of the numbers of test and QA samples. Also, the number of duplicates should be 1 in every 20 samples or less.

19. Page 20, Section 6.0: Typo – additional "of" in first sentence.

References

Bayer, J.M., M.H. Meeuwig, and J.G. Seelye. 2001. *Identification of Larval Pacific Lampreys (Lampetra tridentata), River Lampreys (L. ayresi), and Western Brook Lampreys (L. Richardson) and Thermal Requirements of Early Life History Stages of Lampreys, Annual Report 2000.* United States Geological Survey (USGS) Biological Resources Division,

Western Fisheries Research Center, Columbia River Research Laboratory. Prepared for the U.S. Department of Energy, Bonnevile Power Administration. January.

USEPA. 2002. *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition.* United States Environmental Protection Agency. October.

NOAA appreciates the opportunity to provide these comments. Please let me know if you have any questions.

Sincerely,

Robert Neely
NOAA Coastal Resource Coordinator

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